ANTIOXIDANT CAPACITY AND HEAT DAMAGE OF POWDER PRODUCTS FROM SOUTH AMERICAN PLANTS WITH FUNCTIONAL PROPERTIES

A. BRIZZOLARI[a1], A. BRANDOLINI*[b], P. GLORIO-PAULET[c], and A. HIDALGO*[d]

[a] Department of Health Sciences, Università degli Studi di Milano, Via Di Rudinì 8, 20142 Milan, Italy.

[b]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Unità di Ricerca per la Zootecnia e l'Acquacoltura (CREA-ZA), via Forlani 3, 26866 S. Angelo Lodigiano (LO), Italy.

[c] Universidad Nacional Agraria La Molina, Facultad de Industrias Alimentarias, Departamento de Ingeniería de Alimentos y Productos Agropecuarios. Av. La Molina s/n, Lima 12, Peru.

[d] Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Via Celoria 2, 20133 Milan, Italy.

*Corresponding authors: andrea.brandolini@crea.gov.it; alyssa.hidalgovidal@unimi.it

[1]Present address: DAN Europe Research Division, DAN Europe Foundation, Roseto degli Abruzzi (TE), Italy

Keywords: camu camu; cat's claw; maca; mashua; mesquite; yacon

ABSTRACT

Aim of the study was to evaluate color, total polyphenol content (TPC), antioxidant capacity (ABTS, FRAP, DPPH), reducing sugars and heat damage (furosine, hydroxymethylfurfural, glucosylisomaltol) of 21 commercial powder products obtained from South-American fruits (mesquite, lucuma, camu camu), seeds (amaranth, purple maize), roots and tubers (yacon, maca, mashua, tocosh), bark (cat's claw) and leaves (graviola). TPC and antioxidant capacity were maximum in camu camu and cat's claw powders, and minimum in tocosh, amaranth, lucuma and maca; graviola, mashua, purple maize and mesquite are raw materials with unique antioxidant properties that stand out from the others. Yacon, mashua and lucuma powders had high reducing sugars content (40.9, 34.4 and 21.2 g/100 g DM, respectively) and heat damage (HMF 146.6 mg/kg, furosine 2399.8 and 2228.4 mg/100 g protein, respectively). Overall, camu camu powder and cat's claw were the most interesting products, having high levels of total polyphenols and antioxidant capacity together with very low heat damage.

1. INTRODUCTION

A major threat to human wellbeing is the oxidative stress, an "imbalance between oxidants and antioxidants in favour of the oxidants" (SIES, 1997), which can lead to cellular damage and facilitate the insurgence of cardiovascular and neurodegenerative diseases, diabetes mellitus, cancer and inflammatory illness (UTTARA *et al*., 2009). An effective approach to prevent oxidative stress is to include in the daily diet products rich in antioxidants which can quench the oxygen free radicals, preventing the oxidation of the cell membrane.

Plants and plant-derived ingredients have been used as medical remedies from prehistoric ages, and still are a major source of health-promoting elements. In recent years, the interest in the identification and utilization of plants rich in antioxidant compounds to limit the oxidative stress (ALMEIDA *et al*., 2011; KRISHNAIAH *et al*., 2011) has been steadily growing, because they may behave as preventive medicine. Several authors have reviewed the beneficial uses of underexploited and little-known plant species used in food production but also in traditional medicine (e.g. BIEL *et al*., 2017; CAMPOS *et al*., 2013; CHIRINOS *et al*., 2013; CONTRERAS-CALDERÓN *et al*., 2011; KRISHNAIAH *et al*., 2011). Peru, thanks to its widely diversified climatic zones, is home to a broad array of endemic plants, which show huge differences in the content and type of nutrients and that are potential sources of valuable bioactive compounds (CAMPOS *et al*., 2018).

The antioxidant capacities of plant-derived products vary depending on their content in polyphenols, vitamin C, tocols and carotenoids, (SAURA-CALIXTO and GOÑI, 2006), as well as on the different processing conditions. While in some cases the plant products are consumed fresh, most often they undergo some type of transformation and/or drying to improve shelf life, to lower transport costs and to reach far off consumers (CINAR, 2018). Accordingly, powders from South-American plants with known health-promoting features (Supplementary Table 1) are manufactured by several industries to find new market niches and to foster the consumption of health-promoting natural products. These innovative powder products, obtained from fruits (mesquite, lucuma and camu camu), seeds (amaranth and purple maize), roots and tubers (yacon, maca, mashua and tocosh), bark (cat's claw) and leaves (graviola), are currently used for the preparation or enrichment of infusions, juices, shakes/smoothies, yogurts, desserts, as well as ingredients in cosmetic and pharmaceutical recipes.

The aim of our study was to evaluate some characterstics of these powder products for their possible utilization as enhancing ingredients in wheat-based oven products. To achieve this goal, 21 commercial powder samples of the above-mentioned species were assessed for color, total polyphenol content, antioxidant capacity, reducing sugars and heat damage.

2. MATERIALS AND METHODS

2.1. Samples

The powders analyzed were acquired in 2016 at an industrial fair dedicated to Peruvian export products (Expoalimentaria, Lima, Peru; www.expoalimentariaperu.com) except amaranth, obtained from the Peruvian market, and two maca samples, bought from the Italian market. Several samples (3-5) of each powder product were collected. A detailed list of the products tested is presented in Table 1.

2.2. Physical and chemical analyses

2.2.1. Color

The color coordinates L^* (luminosity), a^* (red-green) and b^* (yellow-blue) of the samples were scored with a tristimulus colorimeter (Chroma meter CR-300, Minolta Italia S.p.A., Italy) using the standard-white reflector plate and illuminant C. Four measurements for each sample were performed.

Table 1. Samples analyzed: species, brands, codes, average dry matter and protein contents (g/100 g).

Product	Species	Brand	Sample	Dry	Protein
Bark					
Cat's claw	*Uncaria tomentosa* L.	A	Cat's claw 1	91.8	0.3
Cat's claw bio	*Uncaria tomentosa* L.	B	Cat's claw 2	92.8	2.7
Cat's claw tea	*Uncaria tomentosa* L.	B	Cat's claw tea	92.5	3.0
Seeds					
Amaranth flour	*Amaranthus caudatus* L.	C	Amaranth FR	90.4	11.5
Amaranth flakes	*Amaranthus caudatus* L.	D	Amaranth FS	90.4	8.8
Purple maize	*Zea mays* L.	B	Purple maize	90.3	7.0
Roots					
Yacon	*Smallanthus sonchifolius*	B	Yacon	87.8	1.8
Maca gluten free	*Lepidium meyenii* Chacon	E	Maca 1	85.9	10.6
Maca bio	*Lepidium meyenii* Chacon	B	Maca 2	87.3	9.0
Maca HP	*Lepidium meyenii* Chacon	B	Maca 3	90.3	8.5
Maca extract	*Lepidium meyenii* Chacon	A	Maca 4	85.4	9.3
Maca	*Lepidium meyenii* Chacon	A	Maca 5	86.6	7.7
Maca root	*Lepidium meyenii* Chacon	F	Maca 6	92.9	12.0
Maca energia	*Lepidium meyenii* Chacon	G	Maca 7	90.4	7.0
Tubers					
Tocosh	*Solanum* spp.	H	Tocosh	83.4	2.2
Mashua	*Tropaeolum tuberosum* Ruiz &	E	Mashua	84.7	9.0
Leaves					
Graviola bio	*Annona muricata* L.	B	Graviola	91.9	10.8
Graviola tea	*Annona muricata* L.	B	Graviola tea	91.0	11.0
Fruits					
Mesquite	*Prosopis* spp.	B	Mesquite	89.3	8.7
Lucuma	*Pouteria lucuma* Ruiz & Pav.	B	Lucuma	88.9	3.4
Camu camu	*Myrciaria dubia* (Kunth)	B	Camu Camu	87.4	5.4

2.2.2. Dry matter and protein content

Dry matter was determined following the gravimetric method, drying 2 g of product at 130 °C for 90 min; protein content was assessed by Kjeldahl (N x 6.25). These and all the following analyses were performed in triple.

2.2.3. Samples preparation for total polyphenols content and antioxidant capacity analysis

All the reagents, of analytical grade, were purchased from Sigma-Aldrich Co. (Milan, Italy). Two different solvents were tested for the extraction of total polyphenols and the evaluation of the antioxidant capacity, i.e. ethanol:H_2O (EtOH:H_2O; 80:20) and methanol:H_2O:acetic acid (MeOH:H_2O:acet; 50:42:8).

Exactly 0.15 g of powdered product were weighed in 2 mL tubes and subjected to three extractions, adding 1 mL of an EtOH:H_2O solution each time. In the first extraction, the samples were stirred with a Vortex (Reax 2000, Meindolph Heidolph, Schwabach, Germany) for 1 min and sonicated (F5200b, Decon, UK) twice for 20 min; in the second extraction the samples were stirred with a Vortex for 1 min, an orbital shaker (Multi-Rotator GRANT-BIO, Cambridge, UK) for 20 min and sonicated for another 20 min; in the third extraction the samples were stirred with a Vortex for 1 min and sonicated for 5 min. After each extraction, the samples were centrifuged with a 4224 centrifuge (ALC Apparecchi per Laboratori Chimici Srl, Milan, Italy) for 5 min at 8048 *g* and all

the supernatants were mixed in a single tube. The extractions were performed at 10 °C and away from light as far as possible.
Following the same procedures, 0.3 g of powdered product underwent three extraction cycles, adding respectively 1.5, 1.5 and 1.0 mL of a MeOH:H_2O:acet solution.

2.2.3.1. Total polyphenol content

Total polyphenol content (TPC) in samples extracted with EtOH:H_2O and MeOH:H_2O:acet was assessed with the Folin-Ciocalteu method as described by BRANDOLINI *et al*. (2013) using a Du-62 Beckman spectrophotometer (Beckman Coulter, Nyon, VD, Switzerland). The TPC, in mg gallic acid equivalent (GAE)/kg DM, was computed from a reference curve obtained from six gallic acid concentrations (range: 0-150 mg/L).

2.2.3.2. Assessment of antioxidant capacity using the ABTS method

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging capacity was analysed as described by YILMAZ *et al*. (2015). A stable stock solution of the ABTS radical cation was prepared by reacting 10 mL of an aqueous solution of 2-2'-azinobis-3-etilenbenzotiazoline 7 mM and 176 µL potassium persulfate 140 mM in the dark at room temperature for 12-16 h. The EtOH:H_2O or MeOH:H_2O:acet extracts (150 µL) were reacted with 5 mL of a diluted ABTS radical solution in ethanol (absorbance: 0.70±0.02 AU at 734 nm); the absorbance was measured at 734 nm, after 6 min at 30 °C, with a V650spectrophotometer (Jasco, Japan), using ethanol as blank. The antioxidant capacity was evaluated as percentage of absorbance decrease (inhibition percentage). A reference curve was built with 11 concentrations (from 0.05 to 0.72 mM) of Trolox. The results are expressed as mmol Trolox equivalents (TE)/kg DM.

2.2.3.3. Assessment of the reduction power using the FRAP method

The ferric reducing antioxidant power (FRAP) was determined as described by YILMAZ *et al*. (2015). Briefly, 200 µL of EtOH:H_2O or MeOH:H_2O:acet extracts were mixed with 4.5 mL FRAP reagent. Absorption was measured with a V650 spectrophotometer (Jasco, Japan) at a wavelength of 593 nm after 60 min incubation at 37 °C; acetate buffer 0.3 M pH 3.6 was used as blank. The FRAP reagent, prepared daily, consisted of 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$ (10:1:1 v/v/v). FRAP values were obtained by comparing the results to a calibration curve built with 18 concentrations (0.06 - 0.90 mM) of Trolox. The antioxidant capacity was expressed as mmol TE/kg DM.

2.2.3.4. Assessment of antioxidant capacity using the DPPH method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical cation scavenging capacity of EtOH:H_2O and MeOH:H_2O:acet extracts was evaluated according to BRANDOLINI *et al*. (2013) using a DU-62 spectrophotometer (Beckman, USA). For each extract five different dilutions were analysed. A dose-response line was computed for each sample and the powder quantity needed to scavenge 50% of the radical (I50) was determined. A reference regression line was computed for the antioxidant Trolox, with concentrations between 3 and 50 µM. The antioxidant capacity was expressed as ratio between I50 of Trolox and I50 of the sample, i.e. mmol TE/kg DM.

2.2.4. Sugars content

Fructose, glucose, maltose and sucrose were assessed by HPLC, following HIDALGO and BRANDOLINI (2011). For peak quantification, sugars calibration curves were constructed using 15 different concentrations (between 0 and 155 mg/L) of fructose, 19 different concentrations (between 0 and 428 mg/L) of glucose, 19 different concentrations (between 0 and 385 mg/L) of maltose, and 15 different concentrations (between 0 and 153 mg/L) of sucrose standards (Sigma, St. Louis, MO, USA). The calibration curves, after log transformation, were linear (r^2 = 1.00; $p \leq 0.001$) in the concentration ranges considered. The results are reported as g/100 g DM.

2.2.5. Heat damage indices.

Furosine was determined by HPLC as described by HIDALGO and BRANDOLINI (2011). A calibration curve was built using nine different concentrations (between 0.33 and 5.13 μmol/L of furosine dihydrochloride (NeoMPS, PolyPeptide Laboratories, Strasbourg, France) in 3 N HCl. The calibration curve was linear ($r^2 = 1.00$; $p \leq 0.001$) in the concentration ranges considered. The results are expressed as milligrams of furosine/100 g of protein.

Hydroxymethlfurfural (HMF) and glucosylisomaltol (GLI) were determined following the HPLC method of RUFIÁN-HENARES *et al*. (2008) as described by HIDALGO and BRANDOLINI (2011). For peak quantification, a calibration curve was constructed using 13 different concentrations (between 0 and 6.25 mg/L) of HMF (Safc, St. Louis, MO, USA). The calibration curve was linear ($r^2 = 1.00$; $p \leq 0.001$) in the concentration range considered. GLI quantification was computed considering the response factor of HMF at 280 nm. The results are expressed as mg/kg DM.

2.3. Statistical analysis

The data were processed by one-way analysis of variance (ANOVA) considering the samples as factors. The distribution of the data was checked and, for normalization purposes, L^* and b^* values were squared, while the other parameters were $\log_{10}$-transformed; however, for easier comprehension, in Tables and Figures the original data are reported.

When significant differences were found ($p \leq 0.05$), Fisher's lowest significant difference (LSD) was computed at a 95% significance level. To compare the results of the two solvents used for the preparation of the extracts, the t-test was applied ($p \leq 0.05$). ANOVA, LSD test and t-test were conducted using the statistical program STATGRAPHICS® Centurion. Mean, standard error and coefficient of variation were computed using the program Excel (Microsoft® Office Excel 2007). Principal Components Analysis (PCA), performed considering the mean values of the 21 samples and all the parameters, was carried out with the software The Unscrambler X 10.2 (CAMO software AS, Norway).

3. RESULTS AND DISCUSSION

3.1. Powders color

Supplementary Table 2 shows the average values and the results of the LSD test for the color coordinates L^*, a^*, b^* of the twenty-one samples. The results obtained grouping the samples by species are reported in Fig. 1.

The broad heterogeneity of the samples led to an ANOVA (not presented) showing significant differences for all the parameters. In fact, a preliminary visual control gave the following color characterization: mashua and purple corn were purple; maca, yellow-orange; cat's claw, mesquite and yacon, orange; graviola, green-brown; camu camu, yellow-brown; tocosh, white; amaranth, cream-white; lucuma, ocher.

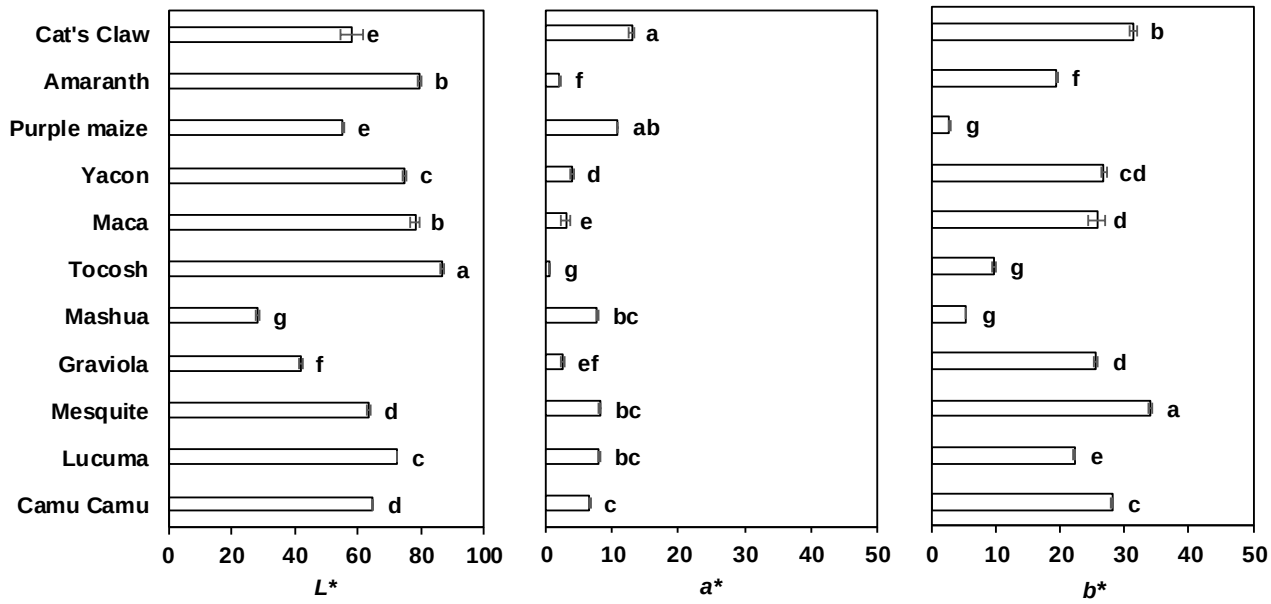

Figure 1. Colour coordinates (*L**, *a**, *b**) of powdered products from 11 species. Different letters indicate significant differences (LSD, $p \leq 0.05$) among species.

The tocosh powder was the brightest (*L**: 86.6), followed by most maca samples (76.2-83.9) and amaranth (78.5-80.3). One maca (maca 4) had an *L** of 72.0, lower than the other maca samples. Camu camu had a *L** like the lyophilized samples (60.45 ± 2.78) and higher than the spouted bed dried samples (36.6-40.8) described by FUJITA *et al*. (2013). Overall, mashua presented the lowest brightness (28.3), followed by graviola (43.3-41.0). Cat's claw and purple corn scored the highest *a** red component values (12.1-13.5 and 10.8, respectively), while tocosh presented the lowest (0.5). The variation among the different maca samples was quite limited, ranging from 1.2 (Maca 7) to 5.1 (Maca 4). Mesquite presented the highest *b** yellow component (34.1), followed by cat's claw (on average 31.6), five maca samples (22.2-26.2) and graviola (on average, 25.6); maca 1 and maca 4 had values different from the other maca (28.3-30.2). Purple corn presented the lowest *b** value, hence the major blue component (2.8), followed by mashua (5.3) and tocosh (9.7). The differences observed between maca samples may be due either to the different treatments utilized for their preparation (ONWUDE *et al*., 2017) or to cultivars with different chromatic characteristics. No information or comparisons for the color components are available in literature.

3.2. Total polyphenol content

Supplementary Table 3 reports the results of TPC, performed on the EtOH:H_2O and MeOH:H_2O:acet extracts, as well as the results of the LSD test comparing the products. The great heterogeneity of the samples led to ANOVAs (not shown) always with significant differences. The average values, obtained by grouping the samples according to the species, are depicted in Fig. 2.

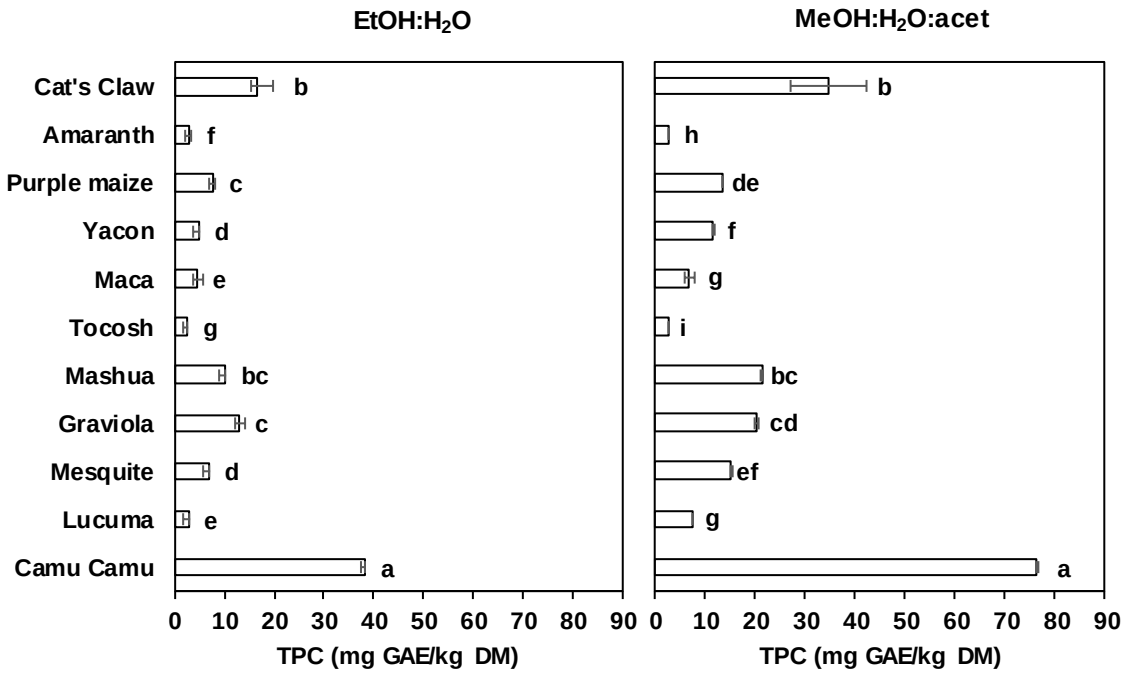

Figure 2. Total polyphenol content (TPC) of the ethanol 80% (EtOH:H_2O) and methanol:H_2O:acetic acid (MeOH:H_2O:acet) extracts of powdered products from 11 species. Different letters indicate significant differences ($p \leq 0.05$) among species.

EtOH:H_2O showed a lower TPC extraction capacity than MeOH:H_2O:acet, but the information provided was similar, as demonstrated by their very high linear coefficient of correlation (r=0.98). TPC was maximum for camu camu (38.3 and 76.4 g GAE/kg DM, respectively), followed by cat's claw 2 (24.2 and 53.6 g GAE/kg DM, respectively); the lowest TPCs were recorded in tocosh (2.5 and 2.8 g GAE/kg DM), amaranth (on average, 3.1 and 2.8 g GAE/kg DM), lucuma (2.8 and 7.5 g GAE/kg DM) and six maca samples (on average, 3.6 and 6.7 g GAE/kg DM). The values generally fell within the range of variation reported in the literature for camu camu (FUJITA *et al.*, 2013), cat's claw (BERLOWSKI *et al.*, 2013; GALVEZ RANILLA *et al.*, 2010), amaranth (REPO-CARRASCO-VALENCIA *et al.*, 2010), lucuma (FUENTEALBA *et al.*, 2016), mesquite (CARDOZO *et al.*, 2010), maca (GALVEZ RANILLA *et al.*, 2010; CAMPOS *et al.*, 2013), mashua (CHIRINOS *et al.*, 2007; CHIRINOS *et al.*, 2013), yacon (CAMPOS *et al.*, 2013), but were lower than those described for graviola frozen pulp (ZIELINSKI *et al.*, 2014). For Peruvian purple maize the information available on TPC is reported in chlorogenic acid equivalent and is not directly comparable to our results, while for tocosh no similar information was found in literature.
The Folin-Ciocalteu method sometimes overstates total phenolics content, because some reducing sugars (e.g. glucose and fructose) may interfere with the results; however, in this research the powders with the highest sugars content (yacon, mashua, lucuma and maca), generally have low TPC; conversely, the two highest TPC values were from camu camu and cat's claw, which showed very low sugars content.

3.3. Antioxidant capacity

The antioxidant capacity of the samples, assessed by the ABTS, FRAP and DPPH tests carried out on the EtOH:H_2O and MeOH:H_2O:acet extracts are shown in Supplementary Table 3, along with the results of the LSD test. The great heterogeneity among samples led to ANOVAs (not presented)

always indicating significant differences, as previously remarked for color and total polyphenols content. The average antioxidant capacities obtained by grouping the samples according to the type of product are presented in Fig. 3.

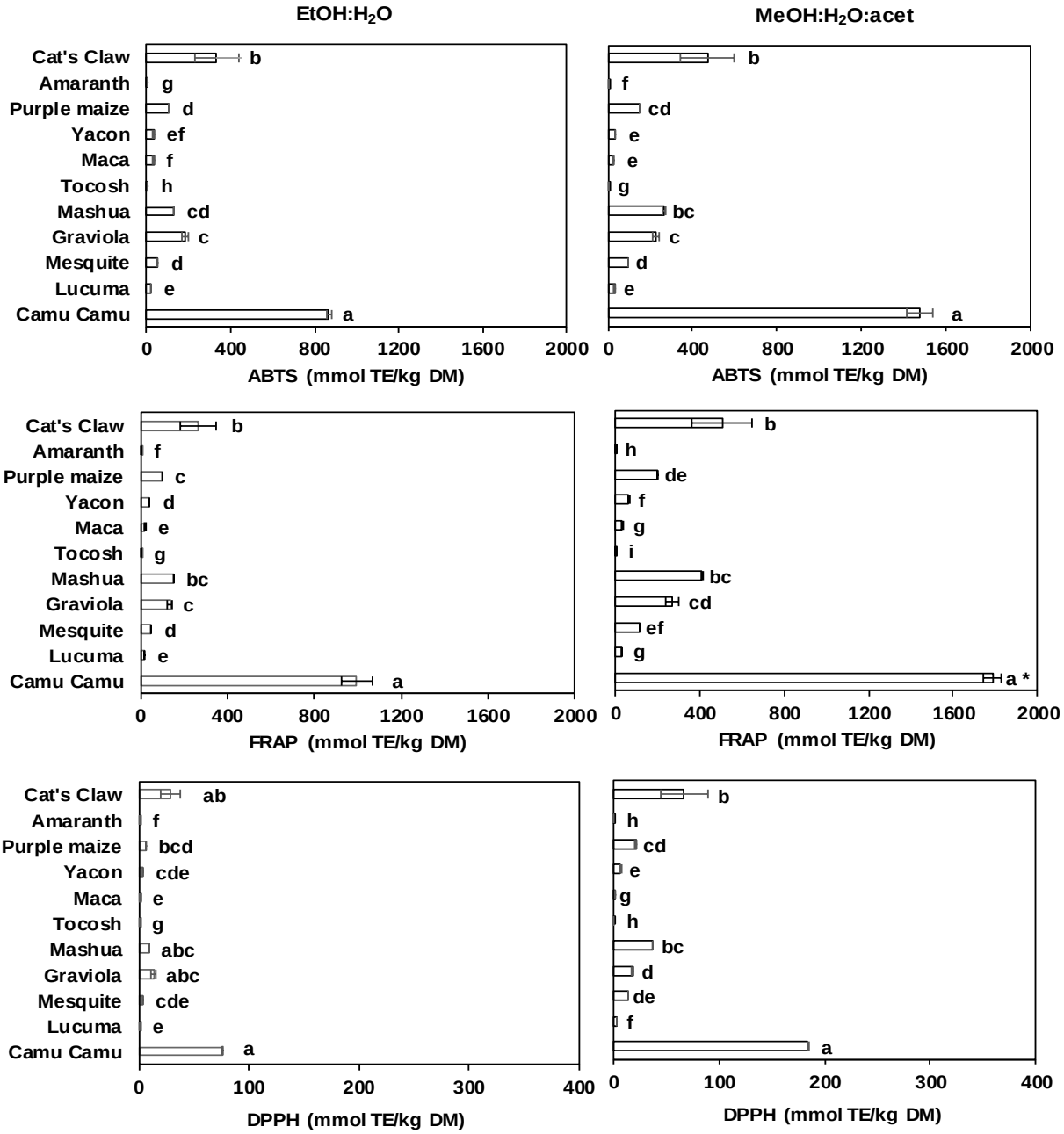

Figure 3. Antioxidant capacity (ABTS, FRAP and DPPH tests,)of the ethanol 80% (EtOH:H_2O) and methanol:H_2O:acetic acid (MeOH:H_2O:acet) extracts of powdered products from 11 species. Different letters indicate significant differences ($p \leq 0.05$) among species.

The ABTS, FRAP and DPPH tests give similar and highly correlated results (r between 0.98 and 1.00 for both EtOH:H_2O and MeOH:H_2O:acet extracts). A higher antioxidant capacity was observed in the MeOH extracts, the exceptions being amaranth (for all three methods), maca and tocosh (ABTS), graviola tea and maca 5 (DPPH). Camu camu, which had the highest TPC concentration (Fig. 2) but also an outstanding vitamin C content (FUJITA *et al*., 2013), showed the highest antioxidant capacity (Fig. 3), followed by cat's claw, graviola, mashua, purple maize and

mesquite. On the other hand, tocosh, amaranth, yacon, maca and lucuma had low antioxidant activities, like that of wheat (YILMAZ *et al*., 2015). Comparable results were reported for camu camu (DPPH: 153-185 μmol TE/g FW; CHIRINOS *et al*., 2010), cat's claw (ABTS: 513 mmol TE/kg DM; FRAP: 507 mmol Fe/kg DM; BERLOWSKI *et al*., 2013), graviola (ABTS: about 200 mmol TE/kg DM; BERLOWSKI *et al*., 2013), mashua (ABTS: 24.3-247.7 μmol TE/g DM; DPPH: 23.2-157.1 μmol TE/g DM; CHIRINOS *et al*., 2013), mesquite (ABTS: 57.0-61.6 μmol TE/g DM; CARDOZO *et al*., 2010), yacon (ABTS 23-136 μmol TE/g DM; CAMPOS *et al*., 2012), purple maize (DPPH: 23.1 μmol TE/g DM; CEVALLOS-CASALS and CISNEROS-CEVALLOS, 2003), lucuma (ABTS: 5.6-304.6 μmol TE/g DM; DPPH: 0.7-132.9 μmol TE/g DM; FUENTEALBA *et al*., 2016) and amaranth (ABTS: 3.7 μmol TE/g DM; DPPH: 1.2 μmol TE/g DM; CHIRINOS *et al*., 2013). On the other hand, those of maca were slightly lower than the levels (ABTS: 67 μmol TE/g DM; FRAP: 11 mmol Fe/kg DM) observed by FUENTEALBA *et al*. (2016).

3.4. Sugars content

The ANOVA (not presented) showed the existence of significant differences for sugars content among samples. The average values and the results of the LSD test for the different sugars are reported in Supplementary Table 2. The reducing sugars results obtained grouping the samples by species are presented in Fig. 4.

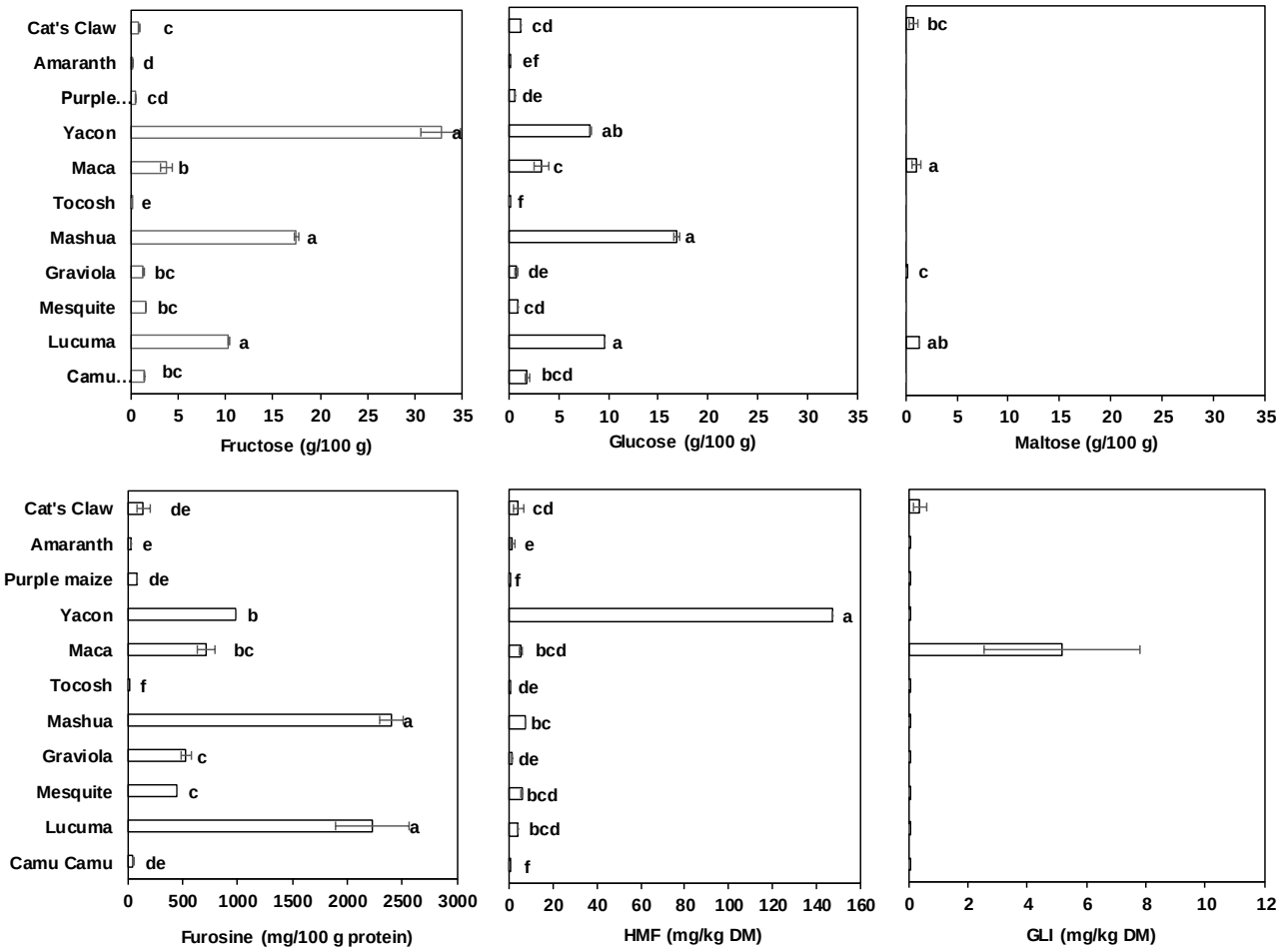

Figure 4. Reducing sugars (fructose, glucose and maltose) and heat damage indices (furosine; hydroxymethylfurfural, HMF; glycosylisomaltol, GLI) of powder products from 11 species. Different letters indicate significant differences ($p \leq 0.05$) among species.

Fructose and glucose were detected in all samples, and were particularly abundant in yacon, mashua and lucuma; maltose was detected only in cat's claw, most maca samples, lucuma and graviola. Overall, yacon (40.9 g/100 g DM), mashua (34.4 g/100 g DM) and lucuma (21.2 g/100 g DM) showed the highest content of reducing sugars which, on the other hand, were almost absent in tocosh (0.11 g/100 g DM) and amaranth (0.30 g/100 g DM). Sucrose (a non-reducing sugar, but a possible source of monosaccharides) was present in moderate quantities in mesquite, maca, mashua, lucuma and yacon (42.5, 25.9, 16.4, 8.30, 8.20 g/100 g DM, respectively) and was very scarce in all the other products. The presence of reducing sugars is important, because they are one of the basic reactants involved in the formation of Amadori products during the Maillard reaction, when exposed to high temperatures (e.g. oven drying, cooking, baking): therefore, higher reducing sugars concentrations forebode higher heat damage during products manufacturing. Among the plants tested, yacon is a well-known source of fructo-oligo-saccharides (CAMPOS *et al*., 2012) and our results are confirmed by the observations (49.2 g/100 g) of SCHER *et al*. (2009). The reducing sugars content found in mashua is analogous to the quantity (6.4-45.3 g/100 g DM, average 28.4 g/100 g DM) reported by GUEVARA-FREIRE *et al*. (2018), while those of maca are slightly lower than the value (13.10±0.17 g/100 g DM) described by RONDÁN-SANABRIA and FINARDI-FILHO (2009), and that of mesquite is slightly inferior to the data (3.17-3.74 g/100 g DM) reported by Cardozo *et al*. (2010) for different *Prosopis* spp. For fructose and glucose content our lucuma results are within the broad range of variation (1.28-12.71 and 2.48-17.37 g/100 g DM) reported by FUENTEALBA *et al.* (2016), and the amaranth ones are very similar to those (0.12 and 0.34 g/100 g DM) presented by GAMEL *et al*. (2006).

3.5. Heat damage

The ANOVA (not presented) showed the existence of significant differences for heat damage among the samples. The average values and the results of the LSD test for heat damage indices, i.e. furosine, GLI and HMF, are reported in Supplementary Table 2. The results obtained grouping the samples by species are reported in Fig. 4.

Non-enzymatic browning in dried products may be influenced by water activity, drying temperature, pH and chemical composition of foods (Sagar Suresh Kumar, 2010). Furosine is an index of the first steps of Maillard reaction, while GLI and HMF are markers of intermediate phases; GLI is formed by the heating of maltose and aminoacids (especially glutamine), while HMF is created not only by degradation of Amadori compounds but also of sugars.

Furosine content was very high in mashua and lucuma (>2000 mg/100 g protein), high in yacon and most maca samples and low in camu camu, amaranth, purple maize as well as in two cat's claw samples. Maca 6 and maca 7 had significantly lower furosine content than the other maca samples. HMF was high only in yacon, but was detected, at lower levels, in several other samples, while GLI was found only in maca 3, maca 6 and cat's claw 1. No Maillard reactions developed in tocosh (a characteristic food, obtained by natural bacterial fermentation of straw-wrapped potatoes kept in running water for several months) as furosine and GLI were lower than the detection limit and HMF was very low, while camu camu, purple maize, amaranths, and cat's claw tea had limited heat damage (low furosine levels and generally below-detection GLI and HMF). Furfural, an indicator of more advanced Maillard reaction stages mainly produced by pentose degradation or thermal degradation of HMF during caramelization, was absent in all samples, even if the method used for GLI and HMF analysis is able to determine its presence.

Since water activity values for all the samples were very similar, ranging between 0.492 and 0.585, and no correlation between heat damage indices and protein content (Table 1) exists, the development of the Maillard reaction seems completely attributable to processing conditions and reducing sugar concentration. The lofty heat damage of yacon (981.3 mg/100 g protein of furosine and 146.6 mg/kg DM of HMF) is justified by its high fructose and glucose content and by the

strong thermal treatment needed to inactivate its highly thermostable polyphenol oxidase enzyme (NEVES DA SILVA, 2007) for a luminous color (Figure 1). These conditions lead to the degradation of the Amadori compounds and to the formation of intermediate compounds (i.e. HMF). The furosine levels of the other samples correlate well to the concentration of reducing sugars (r = 0.93). Thus, mashua, lucuma and most of maca powders with relevant content of reducing sugars have high furosine levels while samples with low reducing sugars content present limited furosine. However, furosine alone is not suitable to completely describe the heat damage of all powder products. The presence of HMF (0.9-11.8 mg/kg DM) in most samples points to an intermediate development of Maillard reaction. The samples with detectable GLI (maca 3, maca 6 and cat's claw 1) showed the highest maltose levels but relatively low reducing sugar concentrations (4.0-7.7 g/100 g DM). The simultaneous formation of HMF suggests higher-than-the-average processing temperatures for these samples. A similar hypothesis can be made for some samples with very low reducing sugar concentrations (0.2-2.5 g/100 g DM) and significant HMF content (1.3-6.0 mg/kg DM).

To the best of our knowledge, no information on heat damage in this type of powder products is available. With reference to other vegetables, furosine contents of 14-262 mg/100 g protein (RUFIÁN-HENARES *et al*., 2013) and of 457-1172 mg/100 g protein (BIGNARDI *et al*., 2016) are reported in sweet pepper and in dried red chili pepper, respectively; similarly, RÍOS-RÍOS *et al*. (2018) describe furosine concentrations of 46.6-110.1 mg/100 g protein in black garlic powder, while HMF levels of 1.3-9.5 mg/kg DM are recorded by SORIA *et al*. (2009) in carrots dried under different conditions.

3.6. Principal components analysis

Fig. 5 depicts the biplots of scores and loadings obtained by the principal components analysis (PCA) performed considering all samples and parameters. PC1 and PC2 describes 46% and 16% of variation (Fig. 5A), while PC3 and PC4 10% and 9% (Fig. 5B); therefore, the initial four PC explain 81% of total variation. The PCA unmistakably separates the different species. PC1, characterized by antioxidant properties, differentiates camu camu and cat's claw along the left side, while PC2, mainly related to heat damage, positions mashua, yacon and lucuma in the upper side (high furosine, HMF, glucose and fructose contents), and maca 3, maca 6, amaranth and tocosh in the bottom side (high *L**, GLI, maltose and protein). PC3 further splits purple maize, graviola, amaranth and tocosh, from mesquite and maca samples, while PC4 divides mashua (top of the plot, characterized by high protein and furosine contents) from yacon (bottom, high HMF and *L**); maca 3 sits alone in a spot defined by high GLI content.

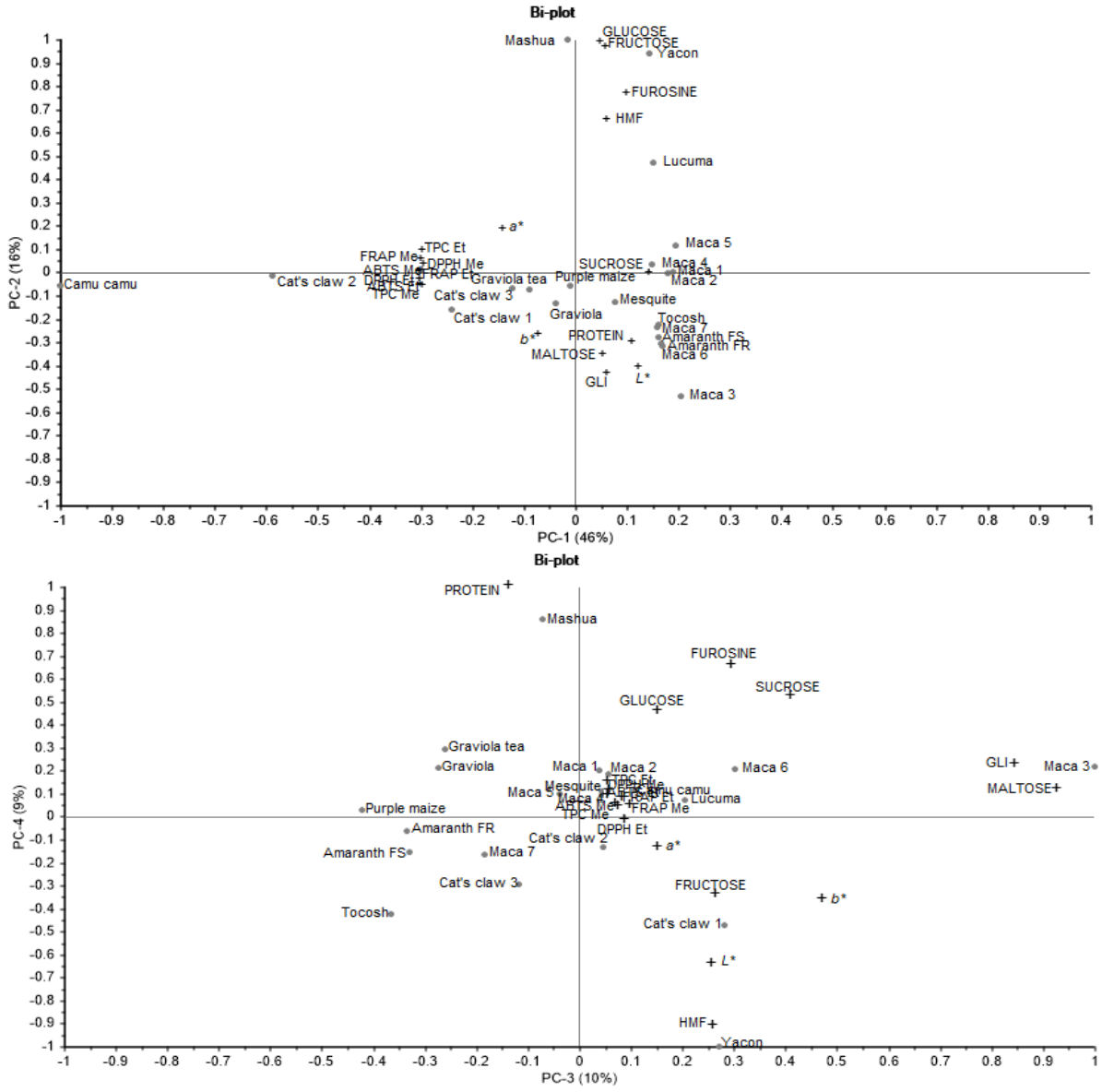

Figure 5. Bi-plot of scores and loads for the first four principal components (PC1 vs. PC2, A; PC3 vs. PC4, B) of the principal component analysis carried out on colour coordinates (*L**, *a**, *b**), protein content, total polyphenol content (TPC) and antioxidant capacity (ABTS, FRAP and DPPH tests) of the ethanol:H2O (Et) and methanol:H_2O:acetic acid (Me) extracts, reducing sugars, furosine, hydroxymethylfurfural (HMF), and glycosylisomaltol (GLI) of 21 powder products.

4. CONCLUSIONS

Our results show that, for the traits analysed, camu camu powder and cat's claw are excellent products, because they have high levels of total polyphenols and antioxidant capacity together with low heat damage. Other interesting products are the powders of graviola, purple maize and mesquite, while the high antioxidant properties of mashua are coupled to severe heat damage.

For an effective use in the food industry, it is necessary to evaluate the stability of the antioxidant capacity of the powders during the manufacturing process and the digestion of innovative high-nutritional-value foods, as well as to assess the sensorial quality of the end products.

ACKNOWLEDGEMENTS

We thank Giulia Malizia and Mattia Magistrelli for their assistance in the lab analyses.

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